

## Fluorescence polarization as a tool for the determination of deoxynivalenol in wheat

C. M. Maragos†\*, M. E. Jolley‡ and M. S. Nasir‡

† Mycotoxin Research Unit, US Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research (USDA-ARS-NCAUR), Peoria, IL 61604, USA

‡ Diachemix Corporation, Grayslake, IL 60030, USA

(Received 24 May 2001; revised 1 August 2001; accepted 29 August 2001)

The mould *Fusarium graminearum* is found worldwide as a pathogen of cereal grains, in particular of wheat and maize, and it produces a mycotoxin known as deoxynivalenol (DON or vomitoxin). Each year, the presence of this compound and related trichothecenes causes substantial losses to agricultural productivity. Rapid methods for the measurement of the toxin in grains are required to monitor and divert effectively contaminated grain from the food supply. A fluorescence polarization (FP) immunoassay using a previously described monoclonal antibody for DON was developed. The assay was based on the competition of unlabeled DON from a sample with a fluorescently tagged DON, DON-fluorescein (DON-FL), for a DON-specific monoclonal antibody in solution. The FP of the tagged DON was increased upon binding with the antibody. In the presence of free toxin, less of the DON-FL was bound and the polarization signal was decreased. The assays were very simple to perform, requiring only mixing of an aqueous extract of wheat with the DON-FL and antibody. The sensitivity of the assay was strongly dependent upon the time between mixing of the sample with the tracer and measurement of the fluorescence polarization, with midpoints for the competition curves ranging from  $0.03 \mu\text{g ml}^{-1}$  with a 15-s incubation to  $>1 \mu\text{g ml}^{-1}$  with a 12-min incubation. Samples of wheat naturally contaminated with DON were evaluated by FP and by an HPLC-UV method, with a good correlation ( $r^2 = 0.97$ ). Although the FP method tended to overestimate DON slightly in

the wheat samples, by  $\sim 20\%$ , the assay was easy to use and very useful for the screening of wheat.

**Keywords:** deoxynivalenol, vomitoxin, fluorescence polarization, immunoassay

### Introduction

Deoxynivalenol (DON or vomitoxin), a mycotoxin produced by *Fusarium graminearum* and *F. culmorum*, can cause disease in several animal species. Swine exposed to sufficient levels of DON demonstrate symptoms that include reduced feed consumption, abdominal distress and in some cases emesis (Rotter *et al.* 1996). DON has been found in a variety of grains and has been detected in wheat, barley and maize worldwide (Jelinek *et al.* 1989). *F. graminearum* is a pathogen of wheat and produces a disease known as head scab, which has caused substantial agricultural losses. The US Food and Drug Administration has established advisory levels for DON in a variety of commodities and food products. The advisory levels range from  $1 \mu\text{g g}^{-1}$  in finished wheat products destined for human consumption to  $5 \mu\text{g g}^{-1}$  in the total diet of ruminating beef, feedlot cattle and chickens. Recently, the Scientific Committee on Food of the European Communities (1999) established a (temporary) tolerable daily intake for DON of  $1 \mu\text{g kg}^{-1}$  body weight.

Commonly used assays for the detection of DON include TLC, HPLC, GC and immunochemical methods such as enzyme-linked immunosorbent assays (ELISAs). DON forms a fluorescent derivative in the presence of aluminium chloride, and this is the basis for several TLC methods (Kamimura *et al.* 1981, Trucksess *et al.* 1986). A fluorescent derivative made with zirconyl nitrate in solution is the basis of a rapid commercial assay for DON (Baxter *et al.* 1985). Detection of the UV absorbance of DON is the basis for several HPLC methods (Lauren and Greenhalgh 1987, Trucksess *et al.* 1996). Recently, HPLC coupled

\*To whom correspondence should be addressed. e-mail: maragocm@ncaur.usda.gov

with mass spectrometry has also been used (Huopalahti *et al.* 1997). The GC methods have employed either electron capture, mass spectrometry or infrared spectroscopy for detection (Scott *et al.* 1993, Mossoba *et al.* 1996, Tacke and Casper 1996).

Immunoassays for DON and the related trichothecenes 3-acetyl-DON (3-AcDON) and 15-acetyl-DON (15-AcDON) have been extensively developed (Casale *et al.* 1988, Mills *et al.* 1990, Usleber *et al.* 1991, Abouzied *et al.* 1993, Nicol *et al.* 1993, Usleber *et al.* 1993, Sinha *et al.* 1995, Schmitt *et al.* 1996, Maragos and McCormick 2000). Very sensitive ELISAs for 3-Ac-DON, with  $IC_{50}$ s of  $\sim 0.1$ – $0.2$  ng ml<sup>-1</sup> have been reported (Usleber *et al.* 1991, Wang *et al.* 1996). A sensitive ELISA has also been produced for 15-Ac-DON, with an  $IC_{50} = 0.35$  ng ml<sup>-1</sup> (Usleber *et al.* 1993) as have ELISAs that rely upon the acetylation of DON before testing (Zhang *et al.* 1986, Xu *et al.* 1988, Schmitt *et al.* 1996). Development of antibodies for DON that do not cross-react with one or more of the acetylated DON derivatives have been problematic. Generally, antibodies that react well with DON also react well with one or more of the acetylated derivatives (Casale *et al.* 1988, Mills *et al.* 1990, Nicol *et al.* 1993, Schmidt *et al.* 1995, Sinha *et al.* 1995, Maragos and McCormick 2000).

Fluorescence polarization (FP) immunoassays have several important differences from ELISAs: detection is not based upon reaction with an enzyme and separation of the bound and free label is not required. Consequently, FP assays can eliminate the separation (wash) required of many ELISAs and can be performed as solution phase assays. In FP assays, the rate of rotation of a fluorophore is measured rather than fluorescence intensity. Polarization is defined by the equation  $P = (I_V - I_H)/(I_V + I_H)$ , where  $I_V$  and  $I_H$  are the intensities of the vertical and horizontal components of the fluorescence, typically expressed as millipolarization units (mP). Polarization is dimensionless, does not depend directly upon the concentration of the fluorophore and has the advantage in that it is minimally affected by solution opacity or colour. It is, however, affected by the size of the molecule, with smaller molecules having higher rates of rotation and lower polarization.

Interaction of an antibody with a toxin-fluorophore conjugate increases the effective size of the fluorophore through the formation of an immunocomplex. The interaction reduces the rate of fluorophore rotation and increases the polarization over that of the unbound fluorophore. In the presence of free toxin,

the formation of the toxin-fluorophore immunocomplex is suppressed, increasing the rate of rotation and reducing polarization. Therefore, similar to many ELISA methods for small molecules, the signal is inversely proportional to toxin content.

The use of FP immunoassay was first described about 40 years ago (Dandliker and Feigen 1961, Haber and Bennett 1962) and, with improvements in instrumentation, is becoming more commonly used as a tool for modern analysis (Checovich *et al.* 1995). Nasir and Jolley (1999) recently reviewed FP immunoassay, and the technique has been extended to the analysis of the fumonisin mycotoxins in maize (Maragos *et al.* 2001). Recently, one of our groups developed three monoclonal antibodies for DON that also cross-react with acetylated DON derivatives (Maragos and McCormick 2000). The objective of the present work was to develop and evaluate an FP immunoassay for DON. This paper describes testing of three monoclonal antibodies for DON in the FP format and the development of sensitive FP assays using one of these antibodies. The DON FP immunoassay was then evaluated by comparing the assay response to that of HPLC methods for DON in naturally contaminated wheat.

## Materials and methods

### Reagents

Except where noted otherwise, deionized water (Nanopure II, Sybron/Barnstead) was used in the preparation of all reagents. All solvents were HPLC grade. Deoxynivalenol (DON), 3-acetyl-deoxynivalenol (3-Ac-DON), 15-acetyl-deoxynivalenol (15-Ac-DON), diacetoxyscirpenol, scirpentriol, trichothecin, T-2 toxin (T-2), T-2 triol, T-2 tetraol, HT-2 toxin (HT-2), neosolaniol, roridin A, verrucarins A and verrucarol were purchased from Sigma Chemical Co. (St Louis, MO, USA). Triacetyl-DON, isotrichodermin, 8-hydroxy-isotrichodermin, trichothecolone and sambucinol were generously provided by Dr Susan McCormick (USDA-NCAUR, Peoria, IL, USA). Nivalenol and fusarenon-X standards were purchased from Wako Pure Chemical Industries, Inc. (Japan). 1,1'-Carbonyldiimidazole was purchased from Aldrich (Milwaukee, WI, USA). Three monoclonal antibodies reactive with DON (clone

reference numbers 1, 4 and 22) were purified from mouse ascites fluid as described (Maragos and McCormick 2000). All other chemicals and solvents were reagent grade or better and purchased from major suppliers.

#### *Preparation of DON-FL tracer*

DON (0.625 mg in 0.1 ml acetone) was mixed with 2 mg 1,1-carbonyldiimidazole and held for 2 h at ambient temperature. Fluoresceinamine isomer II (6-aminofluorescein) solution, 0.1 ml of a 10 mg ml<sup>-1</sup> stock in 0.1 M sodium carbonate (pH 9.5) was added, mixed thoroughly and the mixture held overnight at ambient temperature. The putative DON-FL product was separated by preparative TLC on silica plates developed with chloroform-methanol-acetic acid (90:10:1 v/v/v). The spot ( $R_f = 0.4$ ) was scraped from the plate, shaken with methanol, centrifuged and filtered. This stock solution of DON-FL was diluted 1:100 with PBS (10 mM sodium phosphate, 0.85% sodium chloride, pH 7.4) to prepare a working solution. A further 1:100 dilution of the working solution, a dilution equivalent to that used in the assays, had an intensity comparable with 1 nM fluorescein. In the absence of antibody, the tracer gave a polarization signal of 40–50 mP. The working solution was stored at 2–8°C until use.

#### *Apparatus*

The fluorescence polarization instrument was Sentry FP (Diachemix Corporation, Grayslake, IL, USA). The unit was portable, with power provided from the battery of an attached laptop computer (Gateway, North Sioux City, SD, USA) through a PCMCIA card model DAQCard-500 (National Instruments Corporation, Austin, TX, USA). The unit also has only one moving part: an access door into which the sample cuvette, a cell culture tube, is placed.

#### *Cross-reactivity of Mab 1 in the FP immunoassay*

For cross-reactivity studies, stock solutions of 21 trichothecene mycotoxins were prepared in either acetonitrile, acetonitrile-water or methanol depending upon the solubility characteristics of the toxin.

Once in solution (generally at 1–2 mg ml<sup>-1</sup>) the toxins were diluted further in 10 mM PBS for assay. Many of the trichothecenes reacted poorly with the antibody and were consequently tested at concentrations as high as 20 µg ml<sup>-1</sup>.

#### *Fluorescence polarization (FP) assay*

DON standards were prepared by diluting the DON stock solution with PBS containing 0.1% sodium azide (PBS-A). Antibody solution (1 ml, 0.4 µg) containing 0.1 mg bovine  $\gamma$ -globulin in PBS-A, was placed into a 10 × 75 mm glass culture tube (VWR Scientific, West Chester, PA, USA). DON standard, or sample extract (20 µl), was added and the test solution mixed thoroughly. The test solution was then placed in the fluorometer and used as the blank. The tracer, 10 µl DON-FL working solution, was then added and mixed. The test solution containing tracer was then returned to the fluorometer and the fluorescence polarization signal (mP) measured. For experiments to elucidate the kinetics of the reaction, the FP signal was acquired after holding at ambient temperature for various times from 3 s to 15 min before measurement. For testing of wheat samples and standards (20 µl) were mixed with 1 ml diluted antibody solution in separate tubes and blanked. The tracer (10 µl) was then added to each tube and after holding for a uniform 10 min, the FP signal was measured (Sentry FP software, Diachemix Corp., in Batch Mode). The DON content of naturally contaminated samples was estimated relative to a standard curve of DON in PBS-A (TableCurve software, Jandel Scientific, San Rafael, CA, USA).

#### *Comparison of FP with HPLC-UV for naturally contaminated samples*

Wheat was ground to a very fine consistency and subsamples were removed for assay using either FP or HPLC with single wavelength UV detection (HPLC-UV). The FP assays were conducted at Diachemix while the HPLC-UV assays were conducted at NCAUR. For testing by FP the subsamples (20 g each) were extracted in triplicate with 100 ml water by shaking for 1 h at ambient temperature. The extract was centrifuged for 1 min (Mini Centrifuge, Bel-Art Products, Pequannock, NJ, USA) and the

supernatant solution tested in the FP assay (above) without further purification. Subsamples tested by the HPLC method were extracted with acetonitrile–water (84:16 v/v). Briefly, 25 g was extracted with 100 ml acetonitrile–water by shaking for 1 h at ambient temperature and then the extract was filtered (Whatman 2V; Whatman International Ltd, Maidstone, UK). The extract was further cleaned by passing 4.5 ml through a solid-phase extraction column that binds many impurities but not DON (Romer 225 column, Romer Laboratories, Union, MO, USA), according to the method of Trucksess *et al.* (1996, 1998). Of the eluate, 4 ml, equivalent to 1 g wheat sample, was transferred to a silane-treated vial and dried under a stream of nitrogen gas at 50°C for 1 h. The dried extract was reconstituted with 0.3 ml water–methanol (4:1 v/v), passed through a 0.2 µm syringe filter, and 20 µl injected for separation by reverse phase HPLC with detection at 220 nm. The HPLC system consisted of a Spectra System P4000 pump (Thermo Separations Products, San Jose, CA, USA), a Rheodyne 9125 injector with a 20 µl loop, a reversed-phase guard column (Applied Biosystems, Inc., Foster City, CA, USA, NewGuard RP-18, 3 × 15 mm, 7 µm), a reversed-phase analytical column (TosoHaas, Montgomeryville, PA, USA, ODS-120T, 4.6 mm × 25 cm, 2 µm), a Spectra System UV2000 detector and a SP4270 integrator (Thermo Separations). The mobile phase was a blend of methanol–water as described by Trucksess *et al.* (1998) at a flow rate of 0.7 ml min<sup>-1</sup>. The HPLC method as used was not capable of distinguishing between 3-AcDON and 15-AcDON and was not used to detect these toxins.

## Results and discussion

The FP assays were very simple to perform: the sample and antibody were mixed. This solution was used as a blank, and then the DON–fluorescein tracer (DON–FL) was added. After a short holding time at room temperature, the fluorescence polarization signal (mP) was measured. The interaction of the antibody with the tracer increased the polarization signal. In the presence of unlabeled DON, the tracer and toxin competed for attachment to the antibody, and the signal decreased in relation to the toxin content. Response in this assay format was highly dependent upon both the antibody used and the time for which the sample was held between the addition of the tracer

and the measurement of the signal (hold time). Three murine monoclonal antibodies developed previously for ELISA applications were tested (Maragos and McCormick 2000). Two of these, produced by reference clones 1 and 4, were capable of interacting with the tracer and increasing the polarization signal while the third antibody (#22) did not. Apparently, antibody 22, which was the most sensitive antibody in the competitive direct ELISA format, either did not bind the tracer or did not affect the polarization of the tracer. Although the remaining two clones were less sensitive in the ELISA format, they nevertheless were very sensitive in the FP immunoassay format.

The data in figures 1 and 2 indicate the effect of hold time upon the response of the FP immunoassay to free toxin in buffer for assays using antibodies 1 and 4 respectively. In both cases, the response (mP) at a given toxin concentration increased over time. The rate of the increase was related to the kinetics of the interaction between the antibody and the tracer or DON and the order of addition of the reagents. The toxin was mixed with the antibody before addition of the tracer. This allowed a DON–antibody complex to form. Once the tracer was added, the DON and tracer competed for the available binding sites. The competition is skewed because many of the binding sites are already occupied by toxin, and they only became available to interact with the tracer once the bound

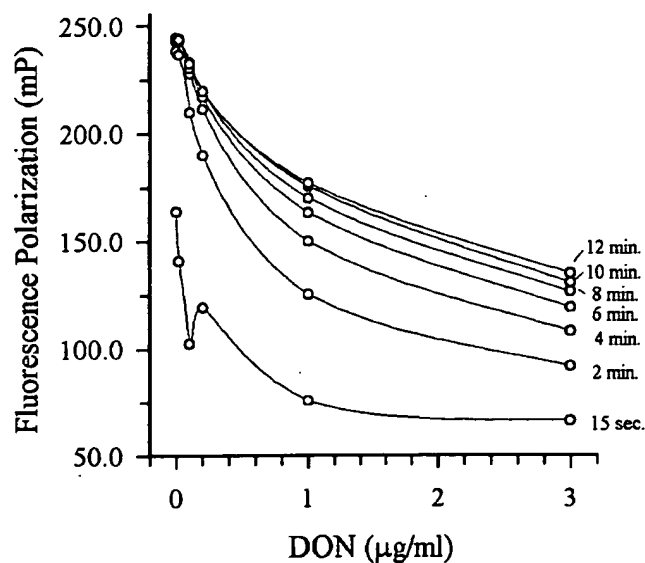


Figure 1. Effect of incubation time upon the response of the FP immunoassay to DON, using antibody 1.

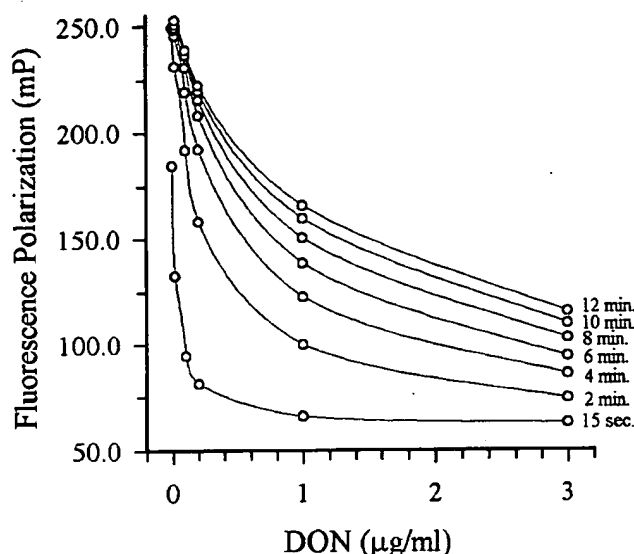


Figure 2. Effect of incubation time on the response of the FP immunoassay to DON, using antibody 4.

DON dissociated from the complex. As a result, the time required for the signal to reach equilibrium was dependent upon the DON concentration. This effect was observed (figures 1 and 2) with rapid equilibration at DON concentrations  $<0.2 \mu\text{g ml}^{-1}$  and slow equilibration at higher DON concentrations.

A benefit for constructing assays with this format was that the assays could be very sensitive and rapid. For example, with antibody 4, the midpoint for the reaction curve occurred at a DON concentration of  $0.03 \mu\text{g ml}^{-1}$  when the reaction was measured after only 15 s. When the reaction was measured after 12 min, the assay was much less sensitive, with a midpoint for the reaction curve of  $\sim 1 \mu\text{g ml}^{-1}$  (figure 2). The disadvantage of using short hold times was the potential that small errors in holding time would translate into large errors in measured toxin content. The potential for such errors would be reduced by holding the samples for a longer period, that is allowing the samples to come to equilibrium before measuring the response. For this reason, a hold time of 10–12 min was used for the studies on cross-reactivity and for the assay of naturally contaminated grain samples, even though the assays were less sensitive with these incubation times. Similarly, antibody 1 was used with the naturally contaminated samples because equilibrium was formed more quickly than with antibody 4.

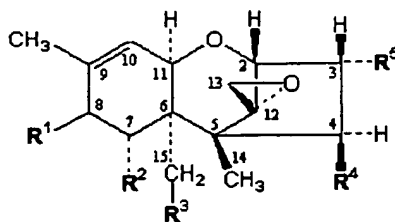
### Cross-reactivity of monoclonal antibody 1

The cross-reactivity of antibody 1 was determined in the FP assay with 21 trichothecenes structurally related to DON (figure 3). Of the mycotoxins tested, only three, 15-AC-DON, DON and HT-2, cross-reacted significantly (table 1 and figure 4). The assay was most sensitive to 15-AC-DON, with an  $\text{IC}_{50} = 540 \text{ ng ml}^{-1}$  when the reaction was held for 12 min before measurement, as described above. HT-2 differs from 15-AC-DON in having an isovaleryl group (instead of a carbonyl group) attached to C-8 and a hydroxyl group at C-4. The response to HT-2, along with the response to DON and the lack of response to most of the trichothecenes modified at C-4, C-8 and C-15, indicates that while the antibody is specific, it can still bind some molecules with modifications in the region between C-4 and C-8. The lack of response to 3-AC-DON and nivalenol (hydroxyl at C-4) indicates the antibody is most specific for the C-3 to C-4 region.

### Naturally contaminated wheat samples

Thirty-four samples of wheat naturally contaminated with DON were analysed by FP immunoassay and HPLC-UV. For these experiments, one subsample was extracted with water for analysis by FP, and another subsample was extracted with acetonitrile-water for analysis by HPLC. Recovery from control wheat (containing  $<0.1 \text{ ppm}$  DON) spiked with 0.5–20 ppm DON averaged  $98 \pm 8\%$  ( $n = 10$ ) using the FP method and  $92 \pm 8\%$  ( $n = 14$ ) using the HPLC-UV method.

As depicted in figure 5, the two methods correlated well, with  $r^2 = 0.967$  for linear regression using the form:  $\text{DON by FP} = 0.302 + 1.21 (\text{DON by HPLC})$ . The fit of the regression highlights two issues. First, the FP assay slightly overestimated DON content for 'negative' wheat samples. A sample negative by HPLC would, in general, give a response equal to  $0.3 \mu\text{g g}^{-1}$  in the FP assay, even though zero falls within the 95% confidence limits of the intercept. Second, there was a systematic overestimation by the FP assay as indicated by the slope of the regression line (1.21). The reason for the overestimation is unknown, but it may be related to the high cross-reactivity of the antibody to 15-AC-DON (table 1)



Common name	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>
Deoxynivalenol	=O	OH	OH	H	OH
3-Acetyl-deoxynivalend	=O	OH	OH	H	OAc <sup>a</sup>
15-Acetyl-deoxynivalenol	=O	OH	OAc	H	OH
Triacetyl-deoxynivalenol	=O	OAc	OAc	H	OAc
Nivalenol	=O	OH	OH	OH	OH
Fusarenon-X	=O	OH	OH	OAc	OH
Trichothecolone	=O	H	H	OH	H
Trichothecin	=O	H	H	X	H
Isotrichodermin	H <sub>2</sub>	H	H	H	OAc
8-hydroxy-isotrichodermin	OH	H	H	H	OAc
Scirpentriol	H <sub>2</sub>	H	OH	OH	OH
Diacetoxyscirpenol	H <sub>2</sub>	H	OAc	OAc	OH
T-2 toxin	ISV	H	OAc	OAc	OH
T-2 triol	ISV	H	OH	OH	OH
T-2 tetraol	OH	H	OH	OH	OH
HT-2 toxin	ISV	H	OAc	OH	OH
Neosolaniol	ISV	H	OAc	OAc	OH
Verrucarol	H <sub>2</sub>	H	OH	OH	H

<sup>a</sup> OAc, ISV and X represent —OCOCH<sub>3</sub>, —OCOCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub> and —OCOCH=CHCH<sub>3</sub>, respectively.

Figure 3. Structure of deoxynivalenol and related trichothecenes.

Table 1. Cross-reactivity of DON monoclonal antibody 1 by FP.

Trichothecene	Average IC <sub>50</sub>	
	±SD (ng ml <sup>-1</sup> ) <sup>a</sup>	% <sup>b</sup>
15-Acetyl-deoxynivalenol	540 ± 24	358
Deoxynivalenol	1930 ± 120	(100)
HT-2 toxin	22 400 ± 2 200	9

<sup>a</sup> IC<sub>50</sub> is the concentration of trichothecene required to give an FP response of 50%, where 100% corresponds to the response of the assay without toxin and 0% represents the response of the assay without antibody. Values are the mean ± 1 SD, using a 12-min incubation for the assay.

<sup>b</sup> Percentage cross-reactivity relative to DON was calculated by dividing the IC<sub>50</sub> of DON by the IC<sub>50</sub> of the indicated trichothecene and multiplying by 100%. Other toxins tested for cross-reactivity, but which were negative (no cross-reactivity at 50 µg ml<sup>-1</sup> were: 3-acetyl-deoxynivalenol, triacetyl-deoxynivalenol, nivalenol, fusarenon-X, trichothecolone, trichothecin, isotrichodermin, 8-hydroxy-isotrichodermin, scirpentriol, diacetoxyscirpenol, T-2 toxin, T-2 triol, T-2 tetraol, neosolaniol, sambucinol, verrucarol, verrucar A and roridin A.

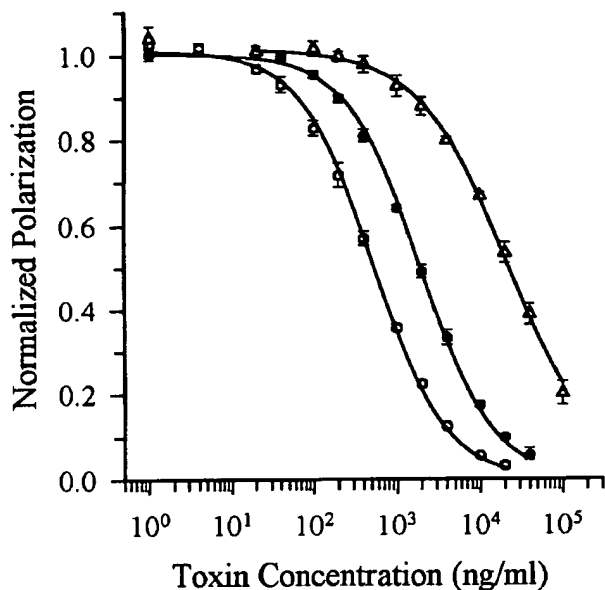


Figure 4. Cross-reactivity of 15-Ac-DON (○), DON (●), and HT-2 (△) in the FP immunoassay using antibody 1.

and the possible presence of 15-AC-DON in the naturally contaminated wheat samples. This overestimation suggests that while the assay may be useful for screening purposes, it should not be used for rigorous quantitation without an adjustment for this discrepancy. Nevertheless, the overall good agreement between the FP and HPLC assays, which were performed at different locations, suggests that wheat designated for use as animal feed can be screened for DON using the FP assay.

## Conclusions

We report the development of rapid fluorescence polarization immunoassays for DON in wheat. The response of the assays was strongly dependent upon the kinetics of the interaction between the toxin or tracer and the antibody used. When configured to have short incubation times (15 s), the assays were very sensitive but required rigorous timing of the incubation step. When configured to have longer incubation times (10–12 min), the assays were less sensitive but were also less susceptible to timing-

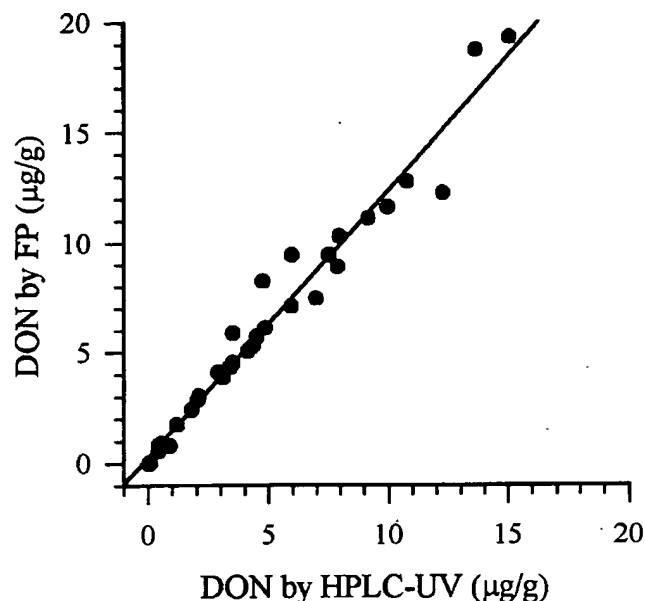


Figure 5. Comparison of DON measurements made by FP and HPLC-UV for naturally contaminated samples of wheat.

induced errors. With wheat, the assay tended to overestimate the DON content. The effect was relatively minor for naturally contaminated wheat samples, where the FP assay agreed well with the HPLC-UV assay (slope = 1.21,  $r^2 = 0.967$ ). The data reported here suggest that FP immunoassays may be feasible for the screening of DON in naturally contaminated wheat. The further development of FP immunoassays for DON using additional combinations of tracer molecules and DON specific antibodies is warranted.

## Acknowledgements

The authors express appreciation to Edward Corrigan, Diachemix Corporation, and Ronald D. Plattner, USDA-ARS-NCAUR, Peoria, IL, for helpful comments and suggestions. Names are necessary to report factually on available data. However, the USDA neither guarantees nor warrants the standard of the products, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

## References

- ABOUZIED, M. M., AZCONA-OLIVERA, J. I., YOSHIZAWA, T., and PESTKA, J. J., 1993, Production of polyclonal antibodies to the trichothecene mycotoxin 4,15-diacetylvalenol with the carrier-adjuvant cholera toxin. *Applied and Environmental Microbiology*, **59**, 1264–1268.
- BAXTER, J. A., TERHUNE, S. J., and CHIA, L. S., 1985, Fluorimetric detection and quantification of deoxynivalenol with zirconyl nitrate-ethylenediamine. *Bulletin of Environmental Contamination and Toxicology*, **34**, 645–649.
- CASALE, W. L., PESTKA, J. J., and HART, L. P., 1988, Enzyme-linked immunosorbent assay employing monoclonal antibody specific for deoxynivalenol (vomitoxin) and several analogues. *Journal of Agricultural and Food Chemistry*, **36**, 663–668.
- CHECOVICH, W. J., BOLGER, R. E., and BURKE, T., 1995, Fluorescence polarization — a new tool for cell and molecular biology. *Nature*, **375**, 254–256.
- DANDLIKER, W. B., and FEIGEN, G. A., 1961, Quantification of the antigen-antibody reaction by the polarization of fluorescence. *Biochemical and Biophysical Research Communications*, **5**, 299–304.
- HABER, E., and BENNETT, J. C., 1962, Polarization of fluorescence as a measure of antigen-antibody interaction. *Proceedings of the National Academy of Sciences, USA*, **48**, 1935–1942.
- HUOPALAHTI, R. P., EBEL, J., JR., and HENION, J. D., 1997, Supercritical fluid extraction of mycotoxins from feeds with analysis by LC/UV and LC/MS. *Journal of Liquid Chromatography and Related Technologies*, **20**, 537–551.
- JELINEK, C. F., POHLAND, A. E., and WOOD, G. E., 1989, Worldwide occurrence of mycotoxins in foods and feeds — an update. *Journal of the Association of Official Analytical Chemists*, **72**, 223–230.
- KAMIMURA, H., NISHIHIMA, M., YASUDA, K., SAITO, K., IBE, A., NAGAYAMA, T., USHIYAMA, H., and NAOI, Y., 1981, Simultaneous detection of several *Fusarium* mycotoxins in cereals, grains, and foodstuffs. *Journal of the Association of Official Analytical Chemists*, **64**, 1067–1073.
- LAUREN, D. R., and GREENHALGH, R., 1987, Simultaneous analysis of nivalenol and deoxynivalenol in cereals by liquid chromatography. *Journal of the Association of Official Analytical Chemists*, **70**, 479–483.
- MARAGOS, C. M., JOLLEY, M. E., PLATTNER, R. D., and NASIR, M. S., 2001, Fluorescence polarization as a means for determination of fumonisins in maize. *Journal of Agricultural and Food Chemistry*, **49**, 596–602.
- MARAGOS, C. M., and MCCORMICK, S. P., 2000, Monoclonal antibodies for the mycotoxins deoxynivalenol and 3-acetyldeoxynivalenol. *Food and Agricultural Immunology*, **12**, 181–192.
- MILLS, E. N. C., ALCOCK, S. M., LEE, H. A., and MORGAN, M. R. A., 1990, An enzyme-linked immunosorbent assay for deoxynivalenol in wheat, utilizing novel hapten derivatization procedures. *Food and Agricultural Immunology*, **2**, 109–118.
- MOSSOBA, M. M., ADAMS, S., ROACH, J. A. G., and TRUCKSESS, M. W., 1996, Analysis of trichothecene mycotoxins in contaminated grains by gas chromatography/matrix isolation/Fourier transform infrared spectroscopy and gas chromatography/mass spectrometry. *Journal of AOAC International*, **79**, 1116–1123.
- NASIR, M. S., and JOLLEY, M. E., 1999, Fluorescence polarization: an analytical tool for immunoassay and drug discovery. *Combinatorial Chemistry and High Throughput Screening*, **2**, 177–190.
- NICOL, M. J., LAUREN, D. R., MILES, C. O., and JONES, W. T., 1993, Production of a monoclonal antibody with specificity for deoxynivalenol, 3-acetyldeoxynivalenol, and 15-acetyldeoxynivalenol. *Food and Agricultural Immunology*, **5**, 199–209.
- PLATTNER, R. D., 1999, HPLC/MS analysis of *Fusarium* mycotoxins, fumonisins and deoxynivalenol. *Natural Toxins*, **7**, 365–370.
- ROTTER, B. A., PRELUSKY, D. B., and PESTKA, J. J., 1996, Toxicology of deoxynivalenol (vomitoxin). *Journal of Toxicology and Environmental Health*, **48**, 1–34.
- SCHMIDT, R., 1995, EZ-Quant, an easy quantitative test for deoxynivalenol. Poster presented at the 109th meeting of the AOAC International, 17–21 September.
- SCHMITT, K., MÄRTLBAUER, E., USLEBER, E., GESSLER, R., LEPSCHY, J., and ABRAMSON, D., 1996, Detection of acetylated deoxynivalenol by enzyme-linked immunosorbent assay. *Immunoassays for Residue Analysis*, edited by R. C. Beier and L. H. Stanker (Washington, DC: American Chemical Society), pp. 314–321.
- SCIENTIFIC COMMITTEE ON FOOD, 1999, Opinion on *Fusarium* Toxins. Part 1: Deoxynivalenol. *Annex VI to the Minutes of the 119th Plenary Meeting* (Brussels: European Commission).
- SCOTT, P. M., KANHERE, S. R., and WEBER, D., 1993, Analysis of Canadian and imported beers for *Fusarium* mycotoxins by gas chromatography-mass spectrometry. *Food Additives and Contaminants*, **10**, 381–389.
- SINHA, R. C., SAVARD, M. E., and LAU, R., 1995, Production of monoclonal antibodies for the specific detection of deoxynivalenol and 15-acetyldeoxynivalenol by ELISA. *Journal of Agricultural and Food Chemistry*, **43**, 1740–1744.
- TACKE, B. K., and CASPER, H. H., 1996, Determination of deoxynivalenol in wheat, barley, and malt by column cleanup and gas chromatography with electron capture detection. *Journal of AOAC International*, **79**, 472–475.
- TRUCKSESS, M. W., FLOOD, M. T., and PAGE, S. W., 1986, Thin layer chromatographic determination of deoxynivalenol in processed grain products. *Journal of the Association of Official Analytical Chemists*, **69**, 35–36.
- TRUCKSESS, M. W., READY, D. E., PENDER, M. K., LIGMOND, C. A., WOOD, G. E., and PAGE, S. W., 1996, Determination and survey of deoxynivalenol in white flour, whole wheat flour, and bran. *Journal of AOAC International*, **79**, 883–887.
- TRUCKSESS, M. W., WOOD, G. E., and CHO, T.-H., 1998, Determination of deoxynivalenol in white flour, whole wheat flour, and bran by solid-phase extraction/liquid chromatography: interlaboratory study. *Journal of AOAC International*, **81**, 880–886.
- USLEBER, E., MÄRTLBAUER, E., DIETRICH, R., and TERPLAN, G., 1991, Direct enzyme-linked immunosorbent assays for the detection of the 8-ketotrichothecene mycotoxins deoxynivalenol, 3-acetyldeoxynivalenol, and 15-acetyldeoxynivalenol in buffer solution. *Journal of Agricultural and Food Chemistry*, **39**, 2091–2095.
- USLEBER, E., SCHNEIDER, E., MÄRTLBAUER, E., and TERPLAN, G., 1993, Two formats of enzyme immunoassay for 15-acetyldeoxynivalenol applied to wheat. *Journal of Agricultural and Food Chemistry*, **41**, 2019–2023.
- WANG, N., KIEREK-JASZCZUK, D., MARQUARDT, R. R., FROHLICH, A. A., and ABRAMSON, D., 1996, Use of a heterologous bridge coating antigen for the immunoassay of 3-acetyldeoxynivalenol in barley. *Journal of Food Protection*, **59**, 525–533.
- XU, Y. C., ZHANG, G. S., and CHU, F. S., 1988, Enzyme-linked immunosorbent assay for deoxynivalenol in corn and wheat. *Journal of the Association of Official Analytical Chemists*, **71**, 945–949.
- ZHANG, G. S., LI, S. W., and CHU, F. S., 1986, Production and characterization of antibody against deoxynivalenol triacetate. *Journal of Food Protection*, **49**, 336–339.